

Quantifying cell mechanical forces as determinants of stem cell fate

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Introduction

Mechanical forces are an important determinant of cell fate. They arise as a consequence of external loading of organs or tissues or due to cell-cell and cell-matrix mechanical interactions. The latter are the consequence of active cellular processes, such as during cell adhesion, migration or division. Experimental studies have shown that substrate stiffness is an important regulator of stem cell fate during in vitro culture, which after in vivo implantation can also influence their contribution to tissue regeneration [1]. While techniques like traction force microscopy (TFM) or extensions like Monolayer Stress Microscopy (MSM) enable to quantify cell-generated forces for single cells or within cell monolayers, quantifying intercellular forces in complex, multicellular systems such as used for tissue engineering, or intracellular forces, remains a challenge. Here we present a computational modelling framework, based on meshless, particle-based methods, that can quantify intercellular and subcellular forces, and which provide useful information on their potential role for (stem) cell fate.

Results and discussion

The framework is able to calculate the mechanical behavior of single, deformable cells as well as multicellular systems consisting of spherical cells, by describing relevant entities as mechanically interacting particles. Depending on the application and considered length scale, a particle can represent the mechanical and geometrical properties of a single cell or a subcellular component (such as part of the cells' membrane or cytoskeleton). Particle motion is governed and calculated by solving a momentum balance. A crucial step is the formulation of equations that capture interacting forces, such as forces due to cell-cell contact, friction, cell division, as well as forces (or equivalent potentials) that govern the deformation of subcellular components.

In previous steps we have demonstrated that this framework can capture the dynamics of cell expansion during microbead culture. Interestingly, we found that even for mechanically identical cells that were seeded randomly on a microbead, strong mechanical heterogeneity in terms of cellular stresses arises at the time of confluency, which was enhanced by an increase of cell-bead adhesion and microbead diameter [2].

More recently we have extended this framework to the study of in vitro micro-aggregation of precursor cells, used for cartilage and bone engineering. Micro-aggregates can be generated in micro-wells consisting of a non-adhesive substrate, such as agarose, thereby preventing the cells from spreading. In such a system cells will first migrate and loosely aggregate, a phase which may be governed by cell-generated chemotactic signals. In a second phase, upon cell-cell contact, condensation will take place, resulting in a closely packed almost spherical aggregate. Both phases can be captured by our model, as shown in figure 1. In the model, migration was modelled by means of a directed random walk (superposition of a random migratory force and a force that is directed towards the source of chemoattractants). Upon cell-cell contact, the adhesion energy was modelled to increase with time, effectively resulting in condensation and displaying a strong increase in cellular stresses. Simulations were calibrated to time lapsed microscopy data of various cell types, such as periosteum-

derived precursor cells and pre-chondrogenic cell lines, such as ATDC5 cells. We could demonstrate that the forces responsible for individual cell migration during aggregation were also responsible for the collective migration of the entire aggregate, which was observed experimentally.

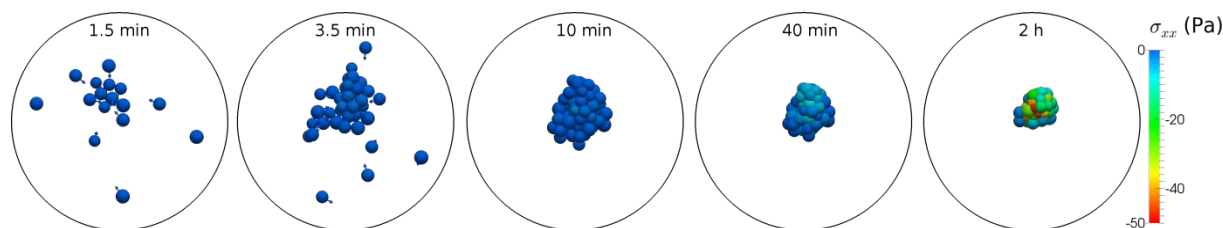


Figure 1: particle-based simulation of the formation of aggregates in micro-wells. Cells have aggregated after 10 minutes and then condensate. After 2 hours an almost spherical aggregate is found. Cells are colour-coded for compressive principal stress (in Pa), arrows indicate migration direction (only visible during aggregation phase).

For applications that involve non-spherical cells and/ or cells that undergo substantial shape changes, a spherical representation of individual cells may not be appropriate. This is e.g. the case during cell migration, a process which is crucial for cell recruitment as part of a regenerative response. For this purpose we have developed a deformable cell model, consisting of particles (nodes) that are interconnected by viscoelastic elements in order to describe the mechanics of the cell cortex. A novel contact algorithm was implemented to quantify the contact pressures and forces for each triangular element (defined by the nodes that serve as vertices) on the cell cortex. In a previous step, this model proved to be a powerful tool to explain the mechanisms of passive cell spreading (i.e. spreading driven by cell-substrate adhesion) [3]. We now extended this model with a description of active cell migration, by defining a protrusive force. This protrusive force was prescribed to those nodes of the triangulation of the cell cortex that are close to the cell-substrate contact. This resulted in a “polymerization pressure” which was biased with the given polarization of the cell. Simulations showed realistic dynamics in terms of cell shape changes, including the formation of a (fluctuating) protrusive front. Apart from the applied protrusive force, it was found that friction between the cell and the substrate played an important role in the cell’s overall migration speed.

These examples demonstrate the potential of the computational framework for quantifying cell mechanical forces and capturing the dynamics in multicellular as well as single cellular systems during various processes, such as division and migration. While the selected examples focused more on tissue engineering applications, the framework is sufficiently versatile to be applied in various contexts of organ and tissue physiology and disease.

References

1. Gilbert P.M., et al. Science, 329, 2010. 2. Smeets B., et al. Comp Meth Biomech Biomed Eng, 16(10), 2013. 3. Odenthal T., et al. PLoS Comput Biol, 9 (10), 2013.

Acknowledgments

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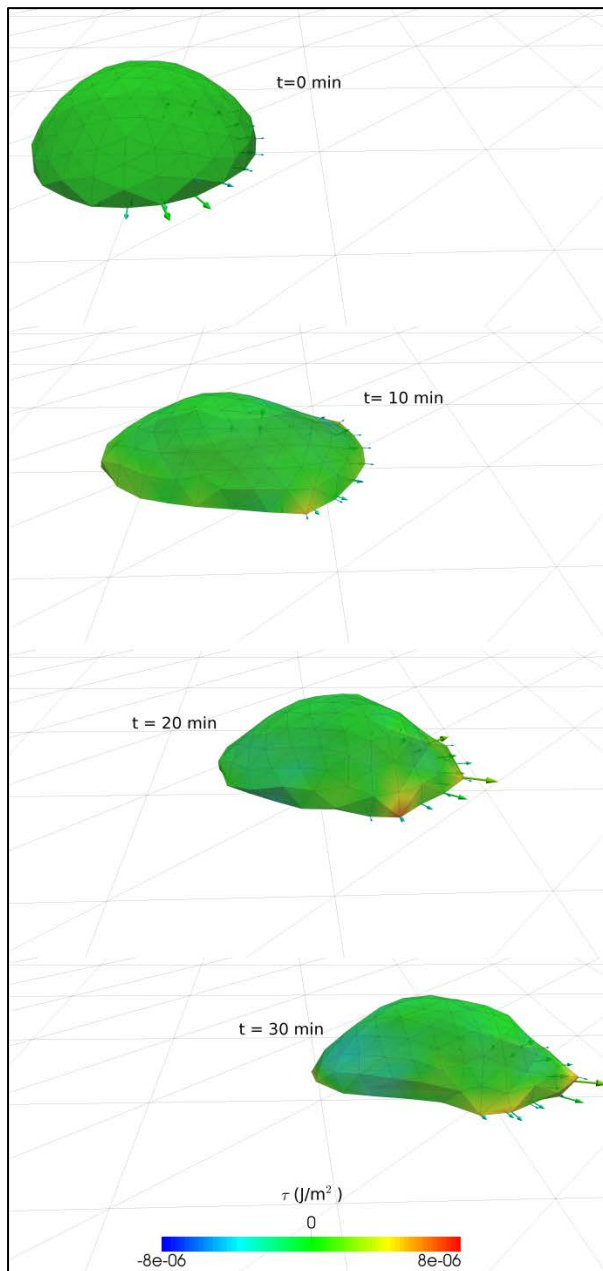


Figure 2: single cell migration on a flat substrate, driven by a distributed, protrusive force (arrows). A 30 minute time period is shown, during which cell shape changes can be appreciated, including the formation of a 'lamellipodium-like' protrusive front. Calculated cortical tensions (in J/m^2) are superimposed on the triangular representation of the cell.